APPROACH TO CONTROL OF POLIOMYELITIS BY IMMUNOLOGICAL METHODS*

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ONE can look upon the entire field of communicable disease with an empirical eye, observing that, in a large number of instances, recovery from one of them is associated with a prolonged resistance, at least to the overt manifestations of that disease. They readily suggest, therefore, that measures can be devised to gain the protective effect without undergoing the dangerous experience of the disease itself. The fact that this has not been uniformly accomplished is evidence that knowledge of the agents and their properties, and of the pathogenesis of their associated diseases, is incomplete. Much of this is attributable to technical difficulties but also to inadequacies of knowledge in the general biological field. Nevertheless, throughout the development of knowledge of infection and immunity one physiological protective mechanism has acquired a dominant position: the production of specific antibodies. Their association with the active resistance of an otherwise susceptible host, their capacity to confer resistance by passive transfer, and numerous other features indicate that they are of prime importance in prevention. And if, despite their continued presence, repeated attacks of the disease are experienced, consideration must be given to the possibility that there is an antigenic multiplicity of the agent, a continuing infection in an intracellular or otherwise protected position, that the antibody being measured is not the right one, or that the antibody is not readily available at the site of infection. In other words, the effectiveness of antibody is related to the nature of the agent and to the pathogenesis of the particular disease.

The role of antibody in immunity to poliomyelitis was for an extended period difficult to clarify and at times was considered to be non-specific, non-protective, subordinate to tissue immunity or to

* Presented at the 27th Graduate Fortnight of The New York Academy of Medicine, October 20, 1954.

Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

physiologic maturation. Rivers[1] summarized the status in 1941: "The role played by neutralizing antibodies in resistance to and recovery from infection (poliomyelitic) is not known." Nevertheless, there was a continuous trend in experimental data to indicate its relation to resistance. In his excellent and complete review of immunization against poliomyelitis, Boyd[2] has concluded:

"1. Studies on immunization against poliomyelitis, for more than three decades, were largely vitiated, through sources of error that were either unrecognized or could not be excluded by any means available at the time. The most serious of these were:
   (a) Lack of quantitative methods for assaying virus and antiserum.
   (b) Ignorance of immunologic diversity among poliomyelitis viruses.
   (c) Use of inappropriate animal species and routes of inoculation for testing the resistance to infection conferred by immunizing procedures."

One can add as a corollary to the last statement that the dominant concepts of pathogenesis were unsatisfactory.

In the past several years there has been advance on a broad front resulting in a consolidation and integration of hitherto spotty observations into a meaningful pattern of information. A major factor in the current advance toward prevention has been the development of evidence leading to present acceptance of poliomyelitis as primarily an infection of the alimentary tract. It has been derived from epidemiological studies of patients, families and communities[3] together with relatively precise reduplication in experimental animals, especially chimpanzees, of man's behavior with the virus.[4, 5] The bulk of human infection appears to be a benign alimentary involvement without penetration of the central nervous system which, nevertheless, gives rise to antibody formation and probable resistance.

Another significant contribution was that of the poliomyelitis virus typing program[6] which was an amplification of work begun in various laboratories. It clearly established the general distribution of three distinct types of poliomyelitis virus but, in addition, led to the convincing quantitative demonstration that monkeys inoculated repeatedly by the intramuscular route developed high levels of type specific antibodies and type specific active immunity even to intracerebral inoculation.

This clarified a very unsettled situation with respect to the significance of antibody and, to my mind, dispelled some of the pessimism concerning the possibility of vaccination. From it came, as well, additional information of the influence of mineral oil adjuvants in the immunization of monkeys to poliomyelitis virus.

Meantime, the use of the Lansing strain for neutralization tests in mice had permitted a better view of the distribution of antibodies in population groups to that type of poliomyelitis virus. It was early pointed out that the frequency of those antibodies was less in persons undergoing clinical disease than in similar unaffected age groups, suggesting a relation of some sort between the presence of those antibodies and resistance to a heterotypic epidemic virus.[7] The use of this procedure for numerous geographic surveys, especially by Paul and his associates,[8a, 8b] Hammon,[9] and others, has demonstrated that the age incidence of poliomyelitis in an area is the reverse of the age incidence of antibodies. Moreover, the accumulated data suggest that the age at which the infections are acquired is generally similar for all three types regardless of the fact that Type I is the major epidemic type of recent years.

Into this setting Enders, Weller and Robbins[10] introduced the notable advance of the cultivation of poliomyelitis virus in tissue cultures, which has revolutionized the immunological and epidemiological study of poliomyelitis. It has provided as well, conditions for varied approaches to immunization against the disease.

Concomitantly, attention was again directed to the question of passive immunization using routes other than the intracerebral for challenge. The fact that gamma globulin would protect monkeys against paralytic disease after administration of virus by peripheral routes was readily established. Bodian[4] has studied the problem in detail in monkeys and chimpanzees, after feeding of virus, with respect to the influence of the globulin on alimentary infection, on the prevention of nervous involvement, and on antibody formation. Speaking of my own reaction, the clear demonstration of the protective effect of gamma globulin in monkeys given relatively large amounts of virus was one of the most significant invitations to vaccination because it was a result of antibody alone without calling on the many other hypothetical explanations which had been invoked at one time or another. When added to this were the systematic studies by Horstmann,[11] and by Bodian[12, 13] of the

occurrence of viremia in cynomolgus monkeys and chimpanzees after feeding of virus, suggesting that viremia may be a deciding factor in the introduction of virus into the nervous system, a coherent pattern of the disease process, highly susceptible to immunological blockade, took form. The fact that this invasion could be prevented by small doses of passive antibody (0.1 ml/k) further heightened the likelihood that procedures which would give moderate levels of antibody could be effective in prevention of paralytic disease while not necessarily eliminating the basic alimentary infection and immunizing process.

Field Trials With Gamma Globulin

Although proposals to use gamma globulin in a controlled investigation of prevention of poliomyelitis had been made earlier (we had conducted a limited institutional study in 1945), the study excellently designed and carried out by Hammon and his associates[14, 15] was the first attempt to get accurate and definitive data on a sufficiently large scale to determine its effectiveness under epidemic conditions in a general community. It is not necessary to review the data here since Doctor Hammon did that for you a year ago. In the period of two to five weeks after administration, seven cases of paralytic poliomyelitis appeared among those receiving gamma globulin and thirty-nine among the controls receiving gelatin. The difference is certainly significant. But the question has been raised whether this is entirely a reduction in incidence resulting from gamma globulin or whether it may represent some enhancement of incidence in the controls as has been demonstrated to occur after certain other forms of inoculation. There is evidence to support this suggestion; nevertheless, a distinct preventive effect of gamma globulin remains.

The original conclusion that gamma globulin brought about a modification of severity of the disease in the first week after inoculation—that is, in the incubation period, seems more doubtful. In fact, Doctor Hammon stated in 1954 that "the data on modification from the 1951-52 experiments do not warrant the conclusions that were drawn in respect to modification" because the possibility could not be reasonably excluded that there might be some unrecognized errors or bias in the data in addition to chance variation.[16] Although the 1953 experience with gamma globulin was not a rigidly controlled investigation, a comparison of the degree of involvement fifty to seventy days after onset

in 184 cases among familial associates who received no globulin and 158 who received it before onset of their illness, revealed no significant difference; nor was a difference detected in the proportion of non-paralytic cases among those who had received gamma globulin before onset.[17] It was suggested that gamma globulin may be ineffective when it is given to patients after they have been infected and the vast majority, if not all, of familial associates of a case may already be infected by the time the first case is diagnosed or by the time inoculations can be given.[18] Data of Brown et al.[19] strongly support this conclusion with respect to the infection. Doctor Hammon[20] has subsequently stated that "one cannot state with assurance that this level of injected antibody has shown no effect upon infection" and after revising the original data on the basis of laboratory identification of cases, he returns to his earlier position with respect to clinical modification with the statement that "these data offer very suggestive, although not conclusive, evidence in support of the use of gamma globulin after exposure has occurred." There is not general concurrence with this opinion. One should hasten to add, however, that failure to demonstrate a major effect in prevention of poliomyelitis among a proportion of familial associates under these conditions does not mean that it might not be possible under other circumstances. The probability also remains that given in sufficient amount and far enough in advance of exposure, it will be preventive. But the evidence at present offers little significant support to its value after infection is instituted and its widespread use in these circumstances is not an efficient procedure. It is conceded, at best, that passive immunization alone is an emergency procedure, but one, nevertheless, which can be of value when employed under opportune conditions, even to serving as a possible cover for immunization through natural infection.

Active Immunization

There are two major approaches being commonly considered for active immunization against poliomyelitis. One, for which there is a large body of support, is that of employing active, modified virus. It can be visualized as an effort to mimic the presumed natural process of establishing an alimentary infection by feeding, although it could be used for parenteral inoculation. Koprowski, Jervis and Norton have made an important step in this direction by feeding to selected children a strain of Type II virus in the form of nervous tissue from cotton

rats.[21a] The virus had a reduced but definite virulence for monkeys by the intracerebral route. Infection was apparently established in forty-seven of the eighty-one subjects, including a proportion who had circulating antibody beforehand. Interestingly, a definite number without antibody to start failed to develop demonstrable infection. Others were reinfected by feeding even in the presence of high antibody titers. Approximately 85 per cent of those without demonstrated antibody developed it within a thirty day period. It is interesting that alimentary infection was established in a low percentage (two of nine) of chimpanzees although all developed antibodies, but in cynomolgus monkeys neither infection nor antibody formation was observed significantly.[21b] These valuable studies clearly illustrate an approach and certain inherent problems, especially that of uncertainty in establishing infection uniformly in those without antibody. A Type I strain adapted to mice has been used by the same authors in three subjects.[21c] Additional investigators have adapted Type III and Type I strains to mice with obvious implications.[22,23]

In his lecture at the Academy in 1953, Cox[24] stated: "We believe that a living, attenuated virus vaccine, comprised of all three major types of poliomyelitis virus propagated in chick embryos, and administered by the oral route, offers the most hopeful, practical and safe procedure to follow for the immunization of children." This is a clear expression of hope supported largely by the fact that a Type II strain has been adapted to eggs, but by no other evidence.

Sabin and his associates[25] have described their extensive studies of the pathogenic characteristics for mice, monkeys and chimpanzees, of variant lines of virus obtained in cultures of monkey kidney cells as a result of spontaneous or forced selection. It constitutes a splendid demonstration of the differences in lines which can be derived and in establishing criteria which may be necessary for selection of lines for further study, particularly those of low neurotropic effect with good antigenic potency. The variation in host reaction of different species is clear. In general, the impression is gained that these variants are not infective or antigenically effective in small doses. It is quite possible that certain of these lines could be used for intramuscular or enteric studies.

Because of the outstanding successes with vaccination against smallpox and yellow fever it is frequently stated that it is only through the use of active virus that an effective and prolonged immunization can be

obtained against virus diseases. This appears to be a generalization which is not fully established.[26] One can point out that they are generalized infections with viruses which are highly uniform antigenically. On the basis of their pathogenetic patterns, they are diseases which should be susceptible to prevention by small amounts of circulating antibody, just as measles and hepatitis are. The latter might well be controlled by inactive virus vaccine, just as they can be by gamma globulin, and one might guess that yellow fever could be also. On the reverse side of the picture, in the case of influenza, ferrets inoculated intranasally with fully virulent virus may some months later respond to reinoculation of the same strain of virus with clinical disease and specific cellular destruction. In man, it has been adequately shown that intranasal inoculation of active influenza virus resulting in clinical disease and antibody formation may not give uniform protection to the same strain four months later. Moreover, evidence indicated that a higher degree of protection was obtained after a similar interval with inactive virus given subcutaneously. Rivers and Ward's[27] experience with vaccinia grown in tissue culture was that perfectly good primary takes were obtained but the degree of immunity was of short duration.

Moreover, the induction of infection with proliferating virus may create injuries whose significance is not readily measurable and, in honesty, are probably insignificant compared with the risk of frank disease. It also carries the risk of contamination with other active viruses. Consider the risks of encephalitis in Western Europe where encephalitis is not an infrequent accompaniment of vaccination. Shope[28] has called attention to the unexpected "breaks" which occur in the veterinary field in association with the use of active virus vaccines, emphasizing the possibility that mutants are not immutable. Nevertheless, certain investigators suggest the possibility that an entire population might disseminate a modified poliomyelitis virus by natural means, much as they do Type II virus at present, with a resultant general immunization. Of course, this would require at least three virus types to be considered. It is rather primitive immunology but rather good biology.

These remarks are in no wise intended to disparage or to discourage studies along this line (in reality it is hoped to encourage them) but more to point out that the thesis is not so firmly established as is sometimes presented and that supposed advantages may have their disadvantages. In the end any vaccine should be safe, practicable and effective and its

effect should be as prolonged as reasonably obtainable. Durability of effect may in any case be influenced by the extent of subsequent natural exposure.

Immunization with inactive virus is based solely upon its capacity to induce antibodies and upon the effectiveness of antibody in preventing or limiting the infection. I have reviewed briefly the experimental evidence with respect to the significance of antibody in poliomyelitis. It has been shown by various investigators that inactivated virus will induce antibody production in human subjects, ordinarily to higher levels than those attained by the administration of gamma globulin. The practical and theoretical risks surrounding the use of an inactivated vaccine prepared for parenteral inoculation from tissue culture of monkey kidney cells has been extensively discussed in the past year. It is essential that virus be inactive according to the best evidence obtainable, that it retain sufficient antigenicity to assure significant antibody levels with a high degree of uniformity and reasonable persistence, and that it be free of serious side effects.

Detailed consideration of these features with respect to the formalinized tissue culture vaccine developed by Salk and proposed for use in the National Foundation for Infantile Paralysis field trial resulted in procedures and criteria which provided a strong guarantee against harmful effects. Moreover, the fact that several hundred children, subsequently more than 7,000, had received similar material and that the antigenicity of all material was to be tested before release gave further assurance of its acceptability.[29] In the course of these investigations Salk had experimented with various routes and schedules of inoculation in increasing numbers of persons and presented evidence that significant antibody levels developed with a high degree of uniformity in persons without demonstrable antibody prior to the administration of vaccine. He had also observed that after seven months most persons tested still possessed antibodies distinctly above the pre-vaccination level. When persons who already possessed some antibody to one or other of the types of virus were given vaccine, or the others were revaccinated at the end of seven months, a sharp "booster" effect was observed.[30,31] This led readily to the suggestion that by these procedures a greater, more prolonged response was possible with inactive virus.

Whether these procedures would provide protection against poliomyelitis and how long it would last was, of course, unknown. This could

be properly determined only by investigation under actual field conditions, for the very behavior of poliomyelitis leaves little middle ground between the small and large study. It is unusual that the final product or schedule is the one with which a beginning is made. Delay could result in a more potent product, antigenically, or a more advantageous schedule of inoculation or other advances. But it would have to be clearly understood that any trial would be an effort to determine whether the vaccine would protect against poliomyelitis under natural conditions – not the distribution of a proven product. It is as well a test of the hypothesis that circulating antibody alone is protective. Should a study be postponed until other approaches were perfected? Numerous approaches and investigations are highly desirable but when safety, applicability and scientific probability of a preparation are demonstrated, a start can be made to answer the basic question – will it prevent – while from many sides studies continue to improve materials and methods. It is here that judgments may honestly differ for if a significant effect is not demonstrable it could be that the principle would be challenged rather than an inadequate product, or vice versa. In addition, consideration must be given to the effect of such an undertaking on the entire field of scientific investigation and on the public confidence upon which it depends.

The next problem is whether a study could be conducted on a sufficiently large scale under conditions which would provide valid data for reaching an objective, and so far as possible, an unbiased conclusion. Provision would need to be made to meet the erratic behavior of the disease with respect to variation in incidence, severity and geographic distribution. Production of safe vaccine in sufficient amounts to embrace a large enough study population would be necessary. There must be assurance that records of sufficient accuracy, uniformity, reliability and completeness could be obtained to provide data of quality. For no matter what his leanings may be, the conclusions of any investigator worth his salt should be governed by demonstrable results. It is here that the importance of adequate controls is evident if a reliable measurement of the effectiveness of the vaccine is to be secured.

After extensive consideration of the requirements, the National Foundation for Infantile Paralysis and its Special Advisory Committee decided that a field trial should be undertaken. The plan originally was to administer vaccine on a voluntary basis to children in the second

grade of school in a large number of counties throughout the United States, selected on the basis of a high incidence of poliomyelitis during the preceding five years. The data indicated that these areas had not only a high five-year average but tended to be consistently high annually. Children in the first and third grades would not be inoculated but would be kept under observation for the occurrence and incidence of poliomyelitis for comparison with the second graders.

However, after further discussion it was agreed that a more rigidly controlled study would also be conducted in a number of areas which were interested in such a plan. In this procedure, equal numbers of children of the first, second and third grades would receive either vaccine or placebo and would be observed thereafter without knowledge of the nature of the inoculum received. While it requires much greater administrative care, this plan clearly permits accumulation and analysis of results on a concealed, coded basis, thus retaining a higher degree of objectivity and avoiding the introduction of bias.

On this basis the Vaccine Evaluation Center was established at the University of Michigan as a completely independent agency which alone would receive records from all study areas and would undertake to obtain complete information and to collate, analyze and interpret the data. It was agreed by all participating areas that reports or estimates would be made only by the Center.

Procedures for the Vaccine Field Trial

I. *Identification of Study Population and Vaccination Records*: It should be pointed out that the program was dependent upon the collaboration of large numbers of lay and professional personnel; the entire program was a remarkable exhibition of common interest and enlightened cooperation in the investigation of a medical problem.

The counties in which the field trial would be undertaken were selected from a list with high incidence by agreement of the state and local health authorities, the medical societies, and the National Foundation for Infantile Paralysis, and with the concurrence of local school or even governmental agencies. Delays occasioned by problems of beginning large scale production and the time consuming safety tests eliminated a number of areas where poliomyelitis had already begun. In the United States the field finally comprised 217 areas in 44 states with approximately 15,000 schools and a total population in the first three

grades of about 1,830,000. There were added areas of three provinces of Canada and of Finland.

Vaccination clinics were begun on April 26, with the participation of the practicing physicians, the state and local health officers, nurses, teachers and principals, and lay volunteers, probably totalling 150,000 persons.

In addition to the inoculation, from approximately 2 per cent of the children – really 40,000 – a sample of blood was to be obtained at the time of the first inoculation and again after the third. The specimens were sent to one of twenty-four virus laboratories which are giving a major part of their effort to the study so that the antibody response of children to the different lots of vaccine as used in the field might be determined. It furnishes information as well of the distribution of antibodies to poliomyelitis viruses at this age in the different parts of the country. Still later, at the end of the season, a third specimen will provide knowledge of the persistence of vaccine effect and perhaps of the frequency of inapparent infection in the study areas. This of itself was an unprecedented undertaking which became possible through development of serological procedures by tissue culture methods, thanks to the fundamental work of Enders, Weller and Robbins.

On receipt of the records from an area they were subjected, at the Center, to checks for completeness with respect to school, grade, and numbers of recorded pupils by the Control and Files Section of our Statistical Division. The individual vaccination record of each child receiving injections was checked against the statements on the class registration schedule for agreement or needed corrections which might even require reference back to the original locality. The record of the lot number of the vaccine or placebo was transferred to the registration schedule, by code, so that the information was not divulged in the process. All the data are coded and prepared for transfer to a punch card which forms a permanent record of each child in the study population.

I shall not detail the massive amount of work involved in these procedures. The process of editing and coding has required the handling of essentially two forms for each child by a temporary staff of over 100 persons, recruited and trained by our own Statistical Division; there are in addition the group supervisors, those who review the finished records for errors or who handle special problems arising in the process. The gross error has been remarkably low – about 1 per cent. The

punching of the machine cards alone is approximately a three months' job, handled by contract through the service office of a tabulating company.

There are now records on something more than 1,830,000 individually identifiable children who constitute the base population for continued observation. Among them are approximately 650,000 children who received inoculations. For all field trial areas in the United States the numbers receiving three inoculations are:

Study Areas		*States*	*Total Study Population**	*Injected Pop.**		
				1st	*2nd*	*3rd*
Placebo	91	11	750	421	411	406
Observed	126	33	1,081	233	230	223
Total	217	44	1,831	654	641	629

* Numbers are given in 1,000's.

The injected population is only that portion of the second graders (or first, second and third graders) who were inoculated, while the total study population under observation comprises all members of the first, second and third grades, irrespective of inoculation or other status. The cases of poliomyelitis occurring in these identified populations constitute the data with which the ability of vaccine to prevent poliomyelitis, especially the paralytic, is to be measured.

II. *Detection and Verification of Cases of Poliomyelitis in Study Population*: It has been said that all we need to know is whether a case is poliomyelitis or not and if it is paralytic. This is precisely what we need to know but we need to know it as precisely as possible. Hence, the second phase of the problem of evaluation is the securing of valid information concerning the occurrence of cases of poliomyelitis among members of the total study population and the collecting of data which establish the diagnosis and the status of the patient with respect to muscular disability and paralysis.

The effort has been to gain information concerning all cases, not some unknown proportion; to establish, or exclude, the diagnosis by use of all available diagnostic procedures; to impose the same standards of uniformity, completeness and reliability of investigation and reporting

with respect to all cases in the study populations regardless of their status in the study. It is apparent that the lines of responsibility and communication are extended and made up of persons of many different qualifications and each is performing a function intimately involved in completion of the total chain of investigation.

It is possible, within the limits of this presentation, to give but a brief outline of the established procedure.

A case of poliomyelitis in the study population is reported by the physician to the local health authority or program director. Telegraphic report is then made to the Evaluation Center. Weekly morbidity reports of cases of all ages are also received at the Center and they are checked for cases in the study population. In addition, records of the Medical Care Program of the National Foundation for Infantile Paralysis are received and examined. There are, thus, multiple sources through which the Center can detect and identify reported cases in study members.

As promptly as possible after a case is reported, a clinical-epidemiological investigation is made and the record is sent through the State Health Department to the Center. At the same time a stool specimen and a specimen of serum is procured and sent to the designated laboratory for virus isolation and serological studies. Simultaneously, one of seventy specially trained physical therapists is notified that a complete muscle evaluation is to be made on the child ten to twenty days after the onset of illness. There is obtained at the same time the interpretation and comment of a physician well qualified in the clinical aspects of poliomyelitis. About one month after onset a second specimen of blood is obtained and sent to the laboratory to permit measure of specific antibody response in comparison with the first specimen. Again, fifty to seventy days after onset the patient is examined by the physical therapist and by the clinical specialist for evidence of disability not detected earlier, or other changes in his muscular status. At the end of nearly three months all of these reports received at the Evaluation Center constitute the basis for determining the diagnosis and the paralytic status of a single patient notified as a case of poliomyelitis. The distribution of these verified cases among the vaccinated and the controls is the evidence with which the effect of vaccination will be measured. Information which may be valuable is contained in additional reports of cases of poliomyelitis in families of a study member and in many instances by laboratory studies which are being made of them.

This simple recitation scarcely expresses the concentration of expert efforts which are contributing to the study of every reported case. Nor does it really present the great attention given by the Evaluation Center to each stage of the process in the effort to obtain prompt and reliable reports. It is readily apparent that the multiple and divided responsibilities resting on persons with many other duties furnish repeated opportunities for irregularities and gaps in procedure and quality. But with insistence on uniformity of procedure with respect to all cases – inoculated or not – in the study age groups, selective bias has largely been avoided and the quality of performance has progressively improved. None of this adequately pictures the extensive operations required of the Staff of the Evaluation Center for the procurement and processing of the information. It is hoped that the effort will provide reliable measurements of the effect of the vaccine under the conditions of study. Our effort has been to keep the data clean so that valid conclusions can be drawn.

SUMMARY

The accumulated evidence points strongly to the probability that the prevention of poliomyelitis by immunological means will be accomplished. The different possibilities which have been proposed present their peculiar problems and possess certain advantages. Continued investigation will undoubtedly clarify their relative effectiveness and practicability. But in each case the effectiveness must be established, not assumed. The current large scale investigation of vaccination with inactivated virus is, in reality, a test of the hypothesis that antibody actively acquired in the absence of infection can, of itself, protect against the disease, an hypothesis which has sound support in laboratory studies and in the field studies of Hammon et al. with gamma globulin.

In an effort to obtain accurate measurement of the effectiveness of preparations of the vaccine developed by Salk for the field trial conducted by the National Foundation for Infantile Paralysis, the Vaccine Evaluation Center has established a thorough registration of the study populations and an intensive investigation combining laboratory, clinical and epidemiologic studies, of each case in the study population. From these data it is expected that reliable conclusions can be drawn.

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